FORM PTO-1390
(REV 11-98)

ATTORNEY DOCKET NUMBER
2861-4003

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S APPLICATION NO (If known see 37 CFR 1.51
TBA 09/787334

INTERNATIONAL APPLICATION	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA99/00844	15 September 1999 (15.09.99)	17 September 1998 (17.09.98)

TITLE OF INVENTION
G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

APPLICANT(S) FOR DO/EO/US
Sylvain CHEMTOB and Krishna G. PERI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S. C. 371 (b) and PCT Articles 22 and 39 (1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))

   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International application into English (35 U.S.C. 371(c)(2)). with oath

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))

   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). Unsigned

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included.

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☐ A FIRST preliminary amendment.

    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or Information:
Published PCT Application Number PCT/CA99/00844 (International Publication Number WO 00/17348) with International Search Report
Notification of Transmittal of the International Preliminary Examination Report (Form PCT/IPEA/416);
International Preliminary Examination Report (Form PCT/IPEA/409) and substitute sheets 1, and 19-21 of PCT application attached.
Notice Informing the Applicant of the Communication of the International Application to the Designated Offices (Form PCT/IB/308)
Statement Under 37 C.F.R. Section 1.821(f) or 1.825(b)
Paper copy of Sequence Listing (3 sheets)
Computer Readable Copy of Sequence Listing

Verified Certification of Express Mailing Date
Return postcard

U.S. APPLICATION NO. (if known, see 37 C F R 1 51)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
TBA 09/787334	PCT/CA99/00844	2861-4003

17. ☒ The following fees are submitted:

	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$1000.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO... $860.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2) paid to USPTO.....$710.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33 (1) - (4).....$690.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1) - (4).....$100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860	
Surcharge of **$130** for furnishing the oath or declaration later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	9 - 20 =	0	X $18.00	$ - 0 -	
Independent claims	1 - 3 =	0	X $80.00	$ - 0 -	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00		

TOTAL OF ABOVE CALCULATIONS =	$ 860.00	
Reduction of ½ for filing by small entity, if applicable. Applicant claims small entity Status.	$ 430.00	
SUBTOTAL =	$ 430.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +	$ - 0 -	
TOTAL NATIONAL FEE =	$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +	$ - 0 -	
TOTAL FEES ENCLOSED	$ 430.00	
	Amount to be refunded:	$
	charged	$

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 13-4500 in the amount of $430.00 to cover the above fees.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-4500, ORDER NO. 2861-4003 A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
Morgan & Finnegan LLP
345 Park Avenue
New York, NY 10154-0053
Telephone: 212-758-4800
Telecopier: 212-751-6849

Mary J. Morry

Registration Number 34,398

609256 1

PATENT
Docket No. 2861-4003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (RO/US)

Applicant(s) : Sylvain CHEMTOB et al. Group Art Unit: TBA

Serial No : 09/787,334 Examiner: TBA

Filed : March 16, 2001

For : G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

PRELIMINARY AMENDMENT

COMMISSIONER FOR PATENTS
BOX PCT
Washington, D.C. 20231

ATTENTION: Shakeel Ahmed

Dear Sir:

Prior to the examination of the above-identified patent application, applicants request entry of the substitute Sequence Listing submitted herewith into the application.

The substitute Sequence Listing is being submitted in response to the Communication mailed April 23, 2001 requesting correction of the Sequence Listing filed March 16, 2001.

REMARKS

The Examiner has requested correction and substitution of the Sequence Listing filed March 16, 2001 by inserting the mandatory features for sections <220> to <223> to explain the "Xaa" for SEQ ID NO. 11. In the accompanying substitute sequence listing, Applicants have corrected the Sequence Listing by inserting sections <220> to <223> explaining that Xaa represents cyclohexyl alanine. Support for this amendment can be found, e.g., in Table 1 on page 14 and on page 14, line 19 of the specification.

This correction in the substitute Sequence Listing is fully supported by the specification and/or figures of the application as originally filed and does not constitute new matter. The substitute Sequence Listing, computer readable form (CRF) of the Sequence Listing (3.5 inch floppy disk) and Statement under 37 C.F.R. § 1.821(f) presently being filed should replace those which were previously filed in the application.

In view of the above remarks, Applicants respectfully request entry of this amendment.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Dated: June 19, 2001

By: ______________________
Mary J. Morry
Registration No. 34,398

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Docket No.: 2861-4003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Sylvain CHEMTOB et al. Group Art Unit: TBA

Serial No : 09/787,334 Examiner: TBA

Filed : March 16, 2001

For : G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

RESPONSE TO NOTIFICATION TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

COMMISSIONER FOR PATENTS
BOX PCT
Washington, D.C. 20231

ATTENTION: Shakeel Ahmed

Dear Sir:

In response to the Communication mailed April 23, 2001, submitted herewith is a paper copy of the substitute sequence listing, a diskette containing the substitute sequence listing in computer readable form, and a Statement under 37 C.F.R. §1.821(f) verifying that the content of the paper and computer readable copies of the substitute sequence listing are the same, as well as a copy of the Notification itself. Also submitted herewith is a Preliminary Amendment directing entry of the sequence listing into the specification.

A response is due June 23, 2001. Therefore, this response is timely filed. However, if that is not the case, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any additional fees that may be required or credit any overpayment to Deposit Account No. 13-4500, Order No. 2861-4003. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,
MORGAN & FINNEGAN, L.L.P.

Dated: June 19, 2001

By: _____
Mary J. Morry
Registration No. 34,398

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345 Park Avenue
New York, New York 10154
(212) 758-4800 Telephone

19 JUN 2001

Patent
Attorney's Docket No. 12667-16US-1 FC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Sylvain Chemtob et al. EXAMINER:

SERIAL NO.: 09/787,334 GROUP ART UNIT:

FILED : March 17, 2001

FOR: G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

Assistant Commissioner for Patents
Washington, D.C. 20231
U.S.A.

STATEMENT UNDER 1.821(f)

Sir:

In connection with this Application a Computer Readable Copy of the Information required under 1.821 is being submitted concurrently together with the sequence listing pages 1/3-3/3.

As required under 1.821(f), the undersigned hereby states that the content of the paper copy which comprises the General Information and the Sequence Listings and the Computer Readable Copy are the same, and that the sequence listing submitted does not introduce new subject matter.

No new matter has been hereby introduced.

This statement is made by a person registered to practice before the U.S. Patent and Trademark Office and as such, a verified statement is not required and is not being submitted.

Respectfully,

By: _____
Christian Cawthorn
Reg. No. 47,352

Date: June 7, 2001

SWABEY OGILVY RENAULT
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SEQUENCE LISTING

<110> HÔPITAL SAINTE-JUSTINE
CHEMTOB, Sylvain
PERI, Krishna G.

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PATENT
Docket No. 2861-4003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Sylvain CHEMTOB and Krishna G. PERI

Serial No. : TBA Group Art Unit: TBA

Filed : March 16, 2001 Examiner: TBA

For : **G PROTEIN-COUPLED RECEPTOR ANTAGONISTS**

Commissioner for Patents
Washington, D.C. 20231

STATEMENT UNDER 37 C.F.R. §1.821(f) OR §1.825(b)

Sir:

I hereby certify that:

[X] The paper Sequence Listing and computer readable form of the Sequence Listing submitted herewith are identical (37 C.F.R. §1.821(f)).

[ ] The paper substitute Sequence Listing and computer readable form of the substitute Sequence Listing submitted herewith are identical. No new matter is included. (37 C.F.R. §1.825(b)).

Respectfully submitted,
MORGAN & FINNEGAN, L.L.P.

Date: March 16, 2001

By: _____
Mary J. Morry
Registration No. 34,398

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G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to development of agonist or antagonist of a G protein-coupled receptor, which bind to the G protein-coupled receptor from the extracellular side in a manner different from that of the natural ligand.

(b) Description of Prior Art

Prostaglandins are derived from the oxygenation of arachidonic acid by prostaglandin synthetases. Prostaglandins mediate a wide variety of physiological actions, such as vasomotricity, sleep/wake cycle, intestinal secretion, lipolysis, glomelular filtration, mast cell degranulation, neurotransmission, platelet aggregation, leuteolysis, myometrial contraction and labor, inflammation and arthritis, patent ductus arteriosus, cell growth and differentiation (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* 46:205-229; Goetzl, E.J. et al., 1995, *FASEB J.* 9:1051-10585). Prostanoids mediate their actions through binding to distinct receptors, which belong to the super family of rhodopsin-like seven transmembrane helical receptors. These receptors are coupled to heterotrimeric G-proteins comprising of α, β and γ subunits which, upon activation, elicit alterations in cell calcium, initiate phosphoinositide hydrolysis or promotion or repression of cyclic adenosine monophosphate synthesis (Strader C. D. et al., 1994, *Ann. Rev. Biochem.* 63:101-132).

Of the five pharmacologically distinct prostanoid receptors for PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$ and TxA_2 and their many isoforms, the receptor for $PGF_{2\alpha}$, also called FP receptor, shows limited tissue distribution, predominantly expressed in corpora leutea, uterine myome-

trium, trabecular meshwork of the eye, and to a lesser extent in vascular smooth muscle. Initiation of labor is marked by tremendous rise in $PGF_{2\alpha}$ levels and increased uterine contractility. The wide spread use of $PGF_{2\alpha}$ analogues to induce labor in veterinary industry points to the primary role of $PGF_{2\alpha}$ and its receptor in parturition. This is underscored by the fact that mice lacking the FP receptor fail to undergo labor (Sugimoto et al., 1997, *Science* **277**:81-83). In face of escalating costs incurred as a result of premature births and associated complications to the neonate, such as intraventricular hemorrhage, bronchopulmonary displasia and periventricular leukomalacia leading to cerebral palsy, prolongation of gestation by arresting premature labor is an effective preventive therapy. The relative success of nonsteroidal anti-inflammatory drugs as a short-term therapy toward prevention of premature labor is based on their inhibitory actions upon the synthesis of prostaglandins, particularly PGE_2 and $PGF_{2\alpha}$. However, inhibition of PGE_2 is associated with serious complications to the fetus such as the closure of ductus arteriosus, renal failure and pulmonary hypertension.

At another level, $PGF_{2\alpha}$ has been attributed a major role in dysmenorrhea, a condition which afflicts 5%-7% of premenopausal women. A pre-menstrual increase in $PGF_{2\alpha}$ levels resulting in myometrial spasms underlies the pathogenesis of this disorder. Lack of effective antagonists of FP receptor for extended therapy hampered the advances in preventing premature labor and associated sequelae, and the design of such antagonists is the subject of this application.

Human FP receptor is a 45 kDa integral membrane glycoprotein, consisting of 359 amino acids and shares only 47% sequence identity with EP_1 receptor, and to a lesser extent with other prostanoid receptors

(Abramovitz et al., 1994, *J. Biol. Chem.* **269**:2632-2636). Binding of $PGF_{2\alpha}$ to FP receptor is followed by the activation of $G_{\alpha q\beta\gamma}$ complex, increased GTP binding by the $G_{\alpha q}$ subunit, stimulation of phospholipase Cβ activity, release of inositol phosphates, increased intracellular calcium and subsequent signal transduction phenomena ultimately leading to smooth muscle contraction (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* **46**:205-229). The FP receptor is the only efficacious target for development of therapeutic drugs since a few G_{α}-proteins catalyze the actions of hundreds of G-protein coupled receptors, thus targets downstream from the receptor are essentially of little use.

Antagonists of FP receptors directed to the ligand binding site could be of limited use since ligand based inhibitors show cross reactivity with other prostanoid receptors. Their efficacy will be compromised in face of tremendous increase in $PGF_{2\alpha}$ concentrations in myometrium at the onset of labor and in menstruation. The high basal activity of the receptors in the absence of ligand limits the use of ligand-based inhibitors.

It would be highly desirable to be provided with agonist or antagonist of FP receptors, which do not crossreact with other prostanoid receptors, and are effective even in the presence of excess ligand.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide agonist or antagonist of FP receptors, which do not crossreact with other prostanoid receptors.

Another aim of the present invention is to provide activators or inhibitors of FP receptors by a novel strategy to target the extracellular domains of the receptor protein.

In accordance with the present invention, there is provided a G protein-coupled receptor agonist or antagonist which specifically binds to the juxtamembrane extracellular structural elements of the G protein-coupled receptor in a manner different from that of the natural ligand, and wherein said agonist or antagonist alter the transduction of an intracellular signal. The G protein-coupled receptor agonist or antagonist may be derived from the amino acid sequence of the receptor.

In accordance with a preferred embodiment of the present invention, the agonist or antagonist does not crossreact with other prostanoid receptors.

The antagonist is effective in the presence of excess ligand.

The agonist or antagonist may preferably comprise an amino acid sequence derived from the first and/or second extracellular loops of prostanoid receptors.

In accordance with another embodiment of the present invention, the antagonists of the present invention comprise amino acid sequences derived from the first and second extracellular loops of prostanoid receptors.

In accordance with a preferred embodiment of the present invention, the G protein-coupled receptor is the prostaglandin $F_{2\alpha}$ receptor (FP receptor).

In accordance with a preferred embodiment of the present invention, the antagonist of the present invention comprises amino acid sequences derived from the prostaglandin $F_{2\alpha}$ receptor.

Preferably, the antagonist include, without limitation, amino acid sequence of the FP receptor selected from the group consisting of ILGHRDYK (PCP-8; SEQ ID NO:1); WEDRFYLL (PCP-10; SEQ ID NO:2); YQDRFYLL

(PCP-14; SEQ ID NO:3); ILAHRDYK (PCP-13.7; SEQ ID NO:4); ILGFRDYK (PCP-13.11; SEQ ID NO:5); ILGHKDYK (PCP-13.13; SEQ ID NO:6); ILGHRNYK (PCP-13.14; SEQ ID NO:7); ILGHQDYK (PCP-13.18; SEQ ID NO:8); ILGHRDY-amide (PCP-13.20; SEQ ID NO:9); ILGHRDYK-amide (PCP-13.21; SEQ ID NO:1); ILGWRDYK (PCP-13.22; SEQ ID NO:10); ILGXRDYK (PCP-13.24; SEQ ID NO:11); SNVLCSIF (PCP-15; SEQ ID NO:12) protein fusions and peptidomimetics thereof; wherein said amino acid sequence contains L- and/or D-amino acid.

In accordance with the present invention, there is provided a peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1 to 12 and wherein said amino acid sequence contains L- and/or D-amino acid, an amino acid sequence with at least about 90% homology to SEQ ID NO:1 to 12, and peptidomimetic thereof.

In accordance with the present invention, there is provided a pharmaceutical composition containing at least a G protein-coupled receptor agonist and antagonist of the present invention, mixture thereof, or functional derivatives thereof in association with a pharmaceutically acceptable carrier.

In accordance with another embodiment of the present invention, there is provided a method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist or functional derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor, thereby hampering uterine contractions.

In accordance with another embodiment of the present invention, there is provided a method for pre-

venting and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist or functional derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor to hamper transduction of a signal thereby reducing the pain associated with contractions.

In accordance with another embodiment of the present invention, there is provided a method of identifying a compound as a G protein-coupled receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, increased/decreased cellular cyclic adenosine monophosphate, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

In accordance with another embodiment of the present invention, there is provided a method of iden-

tifying a compound as a prostaglandin F_2 alpha receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

For the purpose of the present invention the following terms are defined below.

The expression "a G protein-coupled receptor agonist or antagonist" is intended to mean any natural or synthetic compound, peptide protein, antibody, peptidomimetic or small chemical molecules, without limitation, insofar as it can specifically bind to the extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal. More preferably, the agonist or antagonist does not crossreact with other prostanoid receptors.

The expression "functional derivatives" of G protein-coupled receptor agonist or antagonist is intended to mean mimetic compounds and/or structurally unrelated compounds with respect to G protein-coupled

receptor antagonist, which can also specifically bind to the extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal.

The expression "peptidomimetic thereof" is intended to mean any chemical entities, mimetic compounds and/or structurally unrelated compounds with respect to G protein-coupled receptor agonist or antagonist, which can also specifically bind to the extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the inhibitory effects of PCP-8 and PCP-10 on FP receptor function upon stimulation with $PGF_{2\alpha}$ in accordance with the embodiment of the present invention;

Fig. 2A illustrates the effects of PCP-8 and PCP-10 on the diameter of the microvessels of pig retina upon stimulation with either $PGF_{2\alpha}$ or thromboxane A_2 mimetic, U46619;

Fig. 2B illustrates the dose response of $PGF_{2\alpha}$ on the diameter of pig microvessels treated previously with PCP-8 or PCP-10;

Fig. 2C illustrates the effects of thromboxane A_2 mimetic, U46619, on the diameter of pig microvessels treated previously with PCP-8 and PCP-10;

Fig. 3A illustrates the effects of PCP-10 upon spontaneous contractions of uterine smooth muscle;

Fig. 3B illustrates the dose response of prostaglandin $F_{2\alpha}$ in the presence/absence of PCP-8 and_PCP-10 upon uterine smooth muscle contraction; and

Fig. 4 illustrates the reversal of basal tone of bovine myometrium even in the presence of FP receptor ligand, $PGF_{2\alpha}$.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a new class of G protein-coupled receptor antagonists, which bind to the extracellular molecular surface, thus hamper signal transduction.

Also provided is a novel strategy to target the extracellular loops of the receptor which contribute to the structural or functional integrity of the receptor. Antagonists thus bind to cognate elements in the extracellular surface of the receptor and prevent the receptor function by interfering with its signal initiation or transduction.

There is provided proof of selectivity of the antagonists to FP receptor by showing an absence of their effects on a related prostanoid receptor for thromboxane A_2, known as TP receptor which is also involved in smooth muscle contraction.

Preparation of inhibitors

Chemical synthesis of PCP-8 and PCP-10:

All peptides which are 8 amino acids in length were synthesized using F-moc chemistry and solid phase Merrifield method two peptides, PCP-8 and PCP-10. These peptides were purified by HPLC and their purity tested by mass spectroscopy.

In accordance with the present invention, a novel strategy of using peptides derived from the extracellular domains of prostaglandin $F_{2\alpha}$ receptor, FP, to inhibit the signal transduction and the functional consequences of FP receptor. This method could be generalized to all G protein-coupled receptors. Peptides derived from the first and second extracellular loops of FP receptor were found to be effective inhibitors of FP receptor.

The present invention could be readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Effects of peptides, PCP-8 and PCP-10, on ligand-induced phosphoinositide hydrolysis in mammalian cells overexpressing the FP receptor

Both PCP-8 and -10 were tested in HEK293 cells expressing the human FP receptor. For this purpose, HEK 293 cells stably expressing human FP receptor were plated in 12-well plates in DMEM medium containing 10% fetal bovine serum, penicillin (10 U/ml) and streptomycin (10 μg/ml) and cultured in a humidified atmosphere containing 5% CO_2 at 37°C. After the wells were 80% confluent, the cells were labeled with 2 μCi/ml of [^{3}H]-*myo* inositol overnight. Next day, the cells were washed once with PBS, and incubated in 0.5 ml of Kreb's buffer containing 10 mM LiCl and indicated concentrations of PCP peptides for 30 min. $PGF_{2\alpha}$ at 1μM was added to the cells and the incubation was carried out for an additional 30 min. The cells were solubilized with 0.1 N NaOH for 10 min and neutralized with 0.1 N formic acid. The lysates were collected and 1 ml each of methanol and chloroform were sequentially added and vortexed briefly. After centrifugation to separate the phases, inositol phosphates were separated by ion exchange chromatography as described below (Berridge, M.J. et al., 1983, *Biochem. J.* **212**:473-482).

Briefly, the medium was discarded and the IP3 synthesis was stopped by adding 0.6 ml ice-cold methanol. The cells were scraped and collected into polypropylene tubes. Distilled water (0.5 ml) and chloroform (0.6 ml) were added and vigorously vortexed for 2 min. The phases were separated by centrifugation at 6000 x g

for 10 min. The aqueous phase was applied to AG-1X-8™ (Formate form) anion exchange columns (1 ml bed volume) and free inositol was eluted with 10 ml of water, followed by 60 mM ammonium formate in 0.1 M formic acid. Then, the inositol phosphates were eluted with 5 ml of 1.2 M ammonium formate in 0.1 M formic acid. After adding 3 volumes of scintillation cocktail (Optiphase-HiSafe III), the eluates were counted by scintillation spectrophotometry.

The results of these experiments are shown in Fig. 1. Data are expressed as fold stimulation of inositol phosphate synthesis by 1 μM $PGF_{2\alpha}$ compared to the unstimulated controls. Both PCP-8 and -10 at 100 μM potently inhibited inositol phosphate synthesis initiated by the action of $PGF_{2\alpha}$ on FP receptor. The half maximal inhibitory concentrations for both PCP-8 and -10 were slightly less than 100 μM.

EXAMPLE II

Testing PCP peptides in porcine eyecup model of *ex vivo* vasomotricity assay

In order to see if the peptides could inhibit FP function using an *ex vivo* model, we chose porcine eyecup model, an *ex vivo* assay of vascular constriction in porcine retinas which we previously described and validated (Li et al., 1996 J. Pharmacol. Expt. Therapeut. 278: 370-377; Li et al., 1997 Am. J. Physiol. 273: R1283-90; Abran et al., 1997 Am. J. Physiol. 272: R995-1001). Since FP receptor densities in newborn vasculature are minimal due to down regulation by high levels of circulating prostaglandins in the perinatal period, the newborn pigs were treated with a prostaglandin synthetase blocker, ibuprofen (30 mg/Kg of bodyweight/ 8 h for 24 h) to increase the density of the receptors as well as their vasomotor effects. By inhibiting circulating prostaglandins, we were able to show potent

inhibition of FP receptor-mediated second messenger synthesis as well as FP-mediated vascular constriction in this eyecup model.

To prepare eyecups, a circular incision was made 3-4 mm posterior to ora serrata to remove the interior segment and vitreous body with minimal handling of the retina. The remaining eyecup was fixed with pins to a wax base in a 20 ml tissue bath containing 20 ml of Kreb's buffer (pH 7.35-7.45), protease inhibitors, leupetin and aprotinin (10 μg/ml each), and equilibrated with 21% oxygen and 5% carbon dioxide at 37°C. The preparations were allowed to stabilize for 30 min. Peptides at 100 μM were added and incubation was continued for 30 min before the addition of $PGF_{2\alpha}$.

Cumulative concentration-responses of $PGF_{2\alpha}$ and TxA_2 mimetic, U46619, (10^{-10} to 10^{-5} M) curves were constructed separately. To assess the reversibility of the antagonists, the eyecups were thoroughly washed (which would wash away the peptide) with incubation medium and concentration response curves for $PGF_{2\alpha}$ were determined. The outer vessel diameter was recorded with a video camera mounted on a dissecting microscope (Zeiss M 400™) and the responses were quantified by a digital image analyzer (Sigma Scan Software, Jandel Scientific, Corte Madera, CA). Vascular diameter was recorded before and 10 min following the topical application of the agent. Each measurement was repeated three times and showed <1% variability.

The results are shown in Fig. 2. The peptide PCP-10 had no effect on the basal tone (diameter of the microvessel) of the vessel (Fig. 2A; left panels). Addition of 1 μM of $PGF_{2\alpha}$ potently constricted the vessel in the absence of the peptide (middle-top panel), whereas presence of PCP-10 at 100 μM markedly inhibited $PGF_{2\alpha}$-mediated vasoconstriction (middle-bottom panel).

The peptide had no effect on the vasoconstriction effected by 1 μM TxA_2 mimetic, U46619, (right panels) acting on another prostanoid receptor coupled to constriction, namely TP receptor. Similar results were obtained for PCP-8 as well. A dose response of $PGF_{2\alpha}$ on the vascular diameter in the presence/absence of PCP-8 and PCP-10 peptides are presented in Fig. 2B. Both peptides abrogated the vasomotor responses even at concentrations exceeding 1μM of $PGF_{2\alpha}$, suggesting, as expected, that the peptides may be acting in a non-competitive fashion. However, the peptides had no effect on vasoconstriction produced by thromboxane A_2 (Fig. 2C).

Similarly, a peptide derived from the first extracellular loop of FP receptor, PCP-15, inhibited $PGF_{2\alpha}$-induced constriction (10^{-7}M) (88.1% over untreated control; Table 1).

EXAMPLE III

Testing peptide variants of PCP-8 in porcine eyecup model of ex vivo vasomotricity assay

In order to understand the structural requirements of PCP-8 in its inhibitory action on $PGF_{2\alpha}$-induced vasoconstriction, different amino acids in PCP-8 sequence were replaced with other D- or L- amino acids and the resulting peptides were chemically synthesized and tested in porcine eyecup model of *ex vivo* vasomotricity assay. These peptide variants are listed in Table 1.

Table 1

Amino acid sequences of peptide variants of PCP-8 and their inhibitory potency in porcine eyecup model of *ex vivo* vasomotricity assay

Peptide PCP-	%Vasomotor response (of max. constriction)[1]	% inhibition of maximal response[2]	Peptide sequence	SEQ ID NO:
8	50.0	50.0	ilghrdyk	1
10	20.0	80.0	wedrfyll	2
14	36.0	64.0	YQDRFYLL	3
13	20.0	80.0	ILGHRDYK	1
13.7	23.8	76.2	ILAHRDYK	4
13.8	46.8	53.2	ILaHRDYK	4
13.11	13.0	87.0	ILGFRDYK	5
13.13	36.9	63.1	ILGHKDYK	6
13.14	40.3	59.7	ILGHRNYK	7
13.18	30.0	70.0	ILGHQDYK	8
13.20	49.6	50.4	ILGHRDY-amide	9
13.21	46.2	53.8	ILGHRDYK-amide	1
13.22	16.6	83.4	ILGWRDYK	10
13.24	6.2	93.8	ILGXRDYK	11
15	11.9	88.1	SNVLCSIF	12

[1]Percent vasomotor response in the presence of 100 μM peptide is calculated as percent change in average vascular diameter produced by 10^{-7} M $PGF_{2\alpha}$ to the eyecup in the presence of the peptide compared to maximal constriction observed in the absence of the peptide.

[2]Percent inhibition produced by each peptide is calculated as (100-per cent vasomotor response).

Small letters indicate L-amino acids and capital letters indicate D- amino acids. I = isoleucine; L= leucine; G =glycine; H=histidine; R=Arginine; D=Aspartic acid; Y=Tyrosine; K=Lysine; A=Alanine; W= Tryptophan; E=Glutamic acid; F= Phenyl alanine; Q=Glutamine; N=Aspargine; P=Proline; S=Serine; X=Cyclohexyl alanine. Peptides were dissolved in DMSO freshly just before the experiment as 10 mM stocks and added to the eye cups 30 min before the addition of 10^{-7} M $PGF_{2\alpha}$.

A total of 25 variants of PCP-8 were synthesized and the efficacious or potent peptides are listed in Table 1. These peptides incorporate L- to D-amino acid changes, deletions, subtle variations in aromaticity, hydrogen bond donor status as opposed to ionic interactions and hydrophobicity. These peptides were tested at 100 μM concentration in porcine retinal vasomotricity assay and the results are summarized in Table 1.

The results are summarized as follows:

1. Converting all L-amino acids of PCP-8 to D-amino acids (PCP-13) increased the inhibitory potency dramatically. Removal of N-terminal hydrophobic dipeptide sequence from either PCP-8 (PCP-11) or PCP-10 (PCP-12) resulted in significant reduction in the inhibitory action of the peptides.
2. Glycine to alanine (13.7) does not change the activity of PCP-13, whereas change to proline (13.16), L-alanine (13.8), or deletion of the residue (13.17) entirely resulted in loss of activity. Glycine is an important linker residue separating the HRD motif from the IL hydrophobic sequence.
3. HRD-motif is important for the activity of PCP-13. Alanine substitutions (13.1-13.3) or to change to L-configuration (13.4-13.6) resulted loss of inhibitory activity of PCP-13. Aromaticity of His is more important than the positive charge, since H to F (13.11) or W (13.22) or X (13.24), but not to Y (13.23), did not result in significant reduction of peptide inhibitory potency. Side chain length appears to be more critical in case of D residue than R; changing D to E (13.12) resulted in loss of half of the inhibitory activity whereas R to K (13.13) or to Q (13.18) affected the activity of PCP-13 moderately. D to N (13.14) resulted in moderate loss of activity,

whereas a serine substitution (13.19) lead to drastic loss of activity of PCP-13.

4. Deletion of terminal lysine (13.15) or substitution with W (13.9) resulted in complete loss of activity; however, conversion of terminal carboxylate into an amide (13.20 & 13.21) resulted in moderate gain of activity of the peptide inhibitor. Substitution of aromatic residue, Y, with E (13.10) completely abolished the inhibitory potency of PCP-13.

Thus the structure of PCP-13 in D-configuration appears to consists of a N-terminal hydrophobic anchor spaced from the central HRD motif by a glycine residue possibly resulting in a turn conformation of the active peptide; Aromatic and hydrophobic interactions at the carboxy terminus may also add to the potency of PCP-13.

EXAMPLE IV

Testing PCP peptides in porcine uterine strip of *ex vivo* basal contraction assay

In *ex vivo* experiments using porcine uterine strips, the peptides were able to prevent both basal and $PGF_{2\alpha}$-induced contraction.

Uterine tissues from non-pregnant adult pigs were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were set up in organ baths containing Kreb's buffer equilibrated with 21% oxygen at 37°C as we have described (Potvin, W. et al., 1990, *Br. J. Pharmacol.* **100**:341-347; Varma, D.R. and Chemtob, S., 1993, *J. Pharmacol. Expt. Ther.* **265**:1096-1104). Contractions were recorded by force transducers on Grass-polygraph. Strips were incubated with or without 100 µM peptides for 30 min before adding $PGF_{2\alpha}$ in step-wise increments (10^{-9} to 10^{-6} M). Data were expressed as percentage increase over the basal level of average tension (g).

A graph of spontaneous uterine contractions (known to be dependent upon prostanoids, mainly $PGF_{2\alpha}$) in the absence and the presence of 100 μM PCP-8 are shown in Fig. 3A. Addition of peptide to the strips reduced the force of basal contraction. A dose response of $PGF_{2\alpha}$ on uterine contractility in the presence or absence of PCP-8 and PCP-10 peptides is shown in Fig. 3B. More than 60% (PCP-8) and 80% (PCP-10) reduction in uterine contraction was observed in all concentrations of $PGF_{2\alpha}$ tested. Thus, both these peptides reduced spontaneous as well as $PGF_{2\alpha}$-induced contractions in the uterine strips.

EXAMPLE V

Testing PCP peptides in bovine uterine strip of *ex vivo* basal contraction assay

Uterine tissues from non-pregnant adult bovine animals were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were set up in organ baths containing Kreb's buffer equilibrated with 21% oxygen at 37°C as described above. Contractions were recorded on Grass-polygraph by force transducers. Strips were incubated with or without 100 μM peptides before adding $PGF_{2\alpha}$ in step-wise increments (10^{-8} to 10^{-6} M). Data were expressed as change in basal level of average tension (g). The results are shown in Fig. 4. Application of PCP-10 peptide at 100 μM reversed the basal tone (contractile state) of the uterine muscle. Addition of $PGF_{2\alpha}$ up to 10μM did not affect the relaxation produced by PCP-10 suggesting that the effects of PCP peptides are independent of the ligand, which was also shown in the previous results.

While the invention has been described in connection with specific embodiment thereof, it will be understood that it is capable of further modifications

and this application is intended to cover any variations, uses or adaptations of the invention following in general, the principles of the invention and including such departures from the present disclosure as come within the known customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A G protein-coupled receptor antagonist of claim 3, which comprises amino acid sequence of the FP receptor selected from the group consisting of ILGHRDYK (PCP-8; SEQ ID NO:1); WEDRFYLL (PCP-10; SEQ ID NO:2); YQDRFYLL (PCP-14; SEQ ID NO:3); ILAHRDYK (PCP-13.7; SEQ ID NO:4); ILGFRDYK (PCP-13.11; SEQ ID NO:5); ILGHKDYK (PCP-13.13; SEQ ID NO:6); ILGHRNYK (PCP-13.14; SEQ ID NO:7); ILGHQDYK (PCP-13.18; SEQ ID NO:8); ILGHRDY-amide (PCP-13.20; SEQ ID NO:9); ILGHRDYK-amide (PCP-13.21; SEQ ID NO:1); ILGWRDYK (PCP-13.22; SEQ ID NO:10); ILGXRDYK (PCP-13.24; SEQ ID NO:11); SNVLCSIF (PCP-15; SEQ ID NO:12); and functional peptide analogues thereof, wherein X is cyclohexyl alanine.

2. A peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:1 to 12 and wherein said amino acid sequence contains L- and/or D-amino acid, an amino acid sequence with at least about 90% homology to SEQ ID NO:1 to 12.

3. A method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of claim 1.

4. A method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of claim 1.

5. A pharmaceutical composition containing at least a G protein-coupled receptor an antagonist of claim 1, mixture thereof, in association with a pharmaceutically acceptable carrier.

6. A method for determining activity of a compound of claim 1 as a G protein-coupled receptor antagonist capable of binding to the extracellular elements of the said receptor, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound at a concentration of 10^{-10} M to 10^{-3} M to be tested for antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, increased/decreased cellular cyclic adenosine monophosphate, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation.

7. A method for determining activity of a compound of claim 1 as a prostaglandin F_2 alpha receptor antagonist capable of binding to the extracellular elements of the said receptor, comprising the steps of:

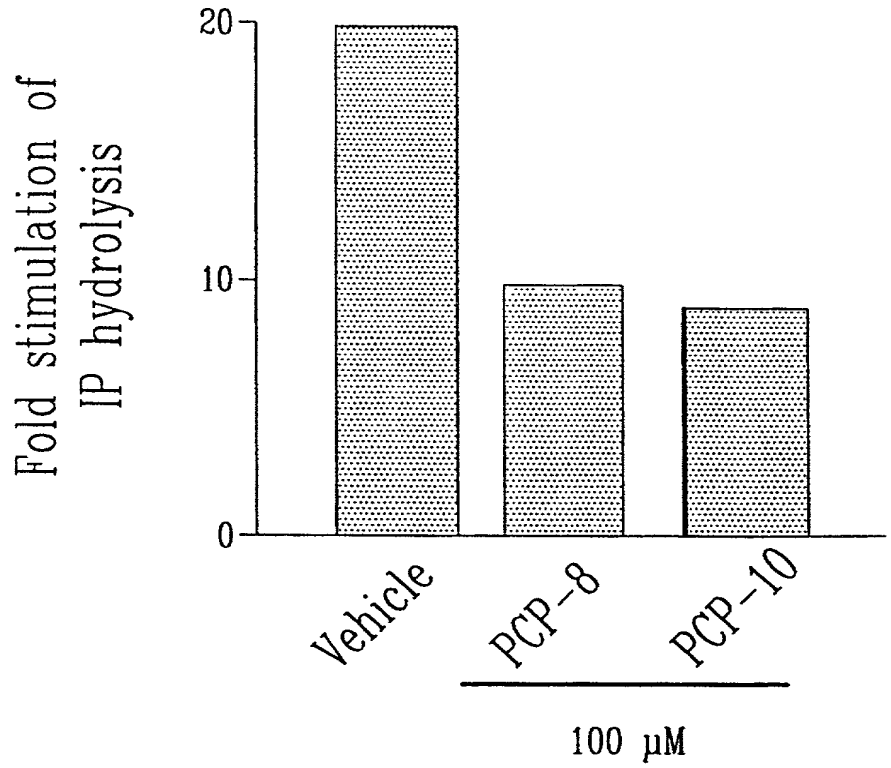
a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound at a concentration of 10^{-10} M to 10^{-3} M to be tested for antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation.

8. The use of a therapeutically effective amount of a G protein-coupled receptor antagonist of claim 1 ~~4~~ for the preparation of a medicament for preventing premature delivery of fetus.

9. The use of a therapeutically effective amount of a G protein-coupled receptor antagonist of claim 1 ~~4~~ for the preparation of a medicament for preventing and/or treating dysmenorrhea.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Vehicle
PCP-8
PCP-10
100 µM

Fig. 1

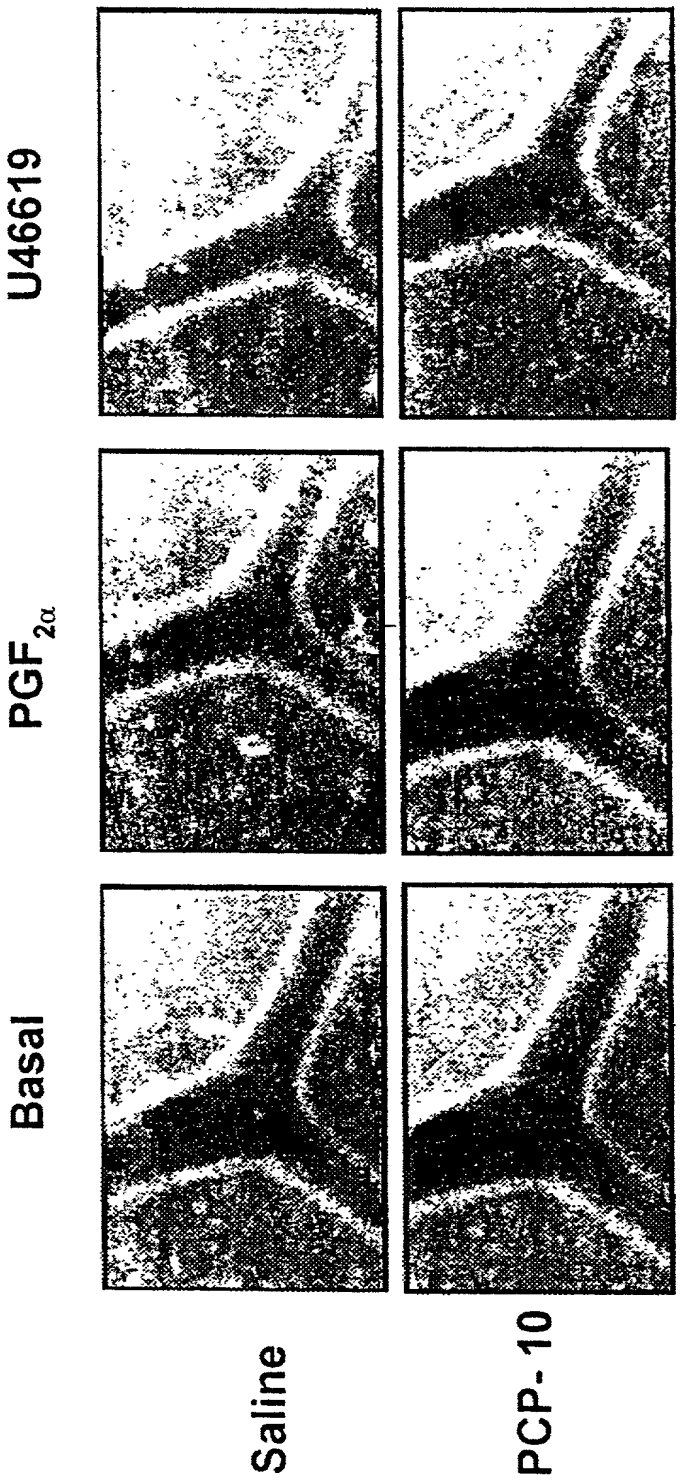
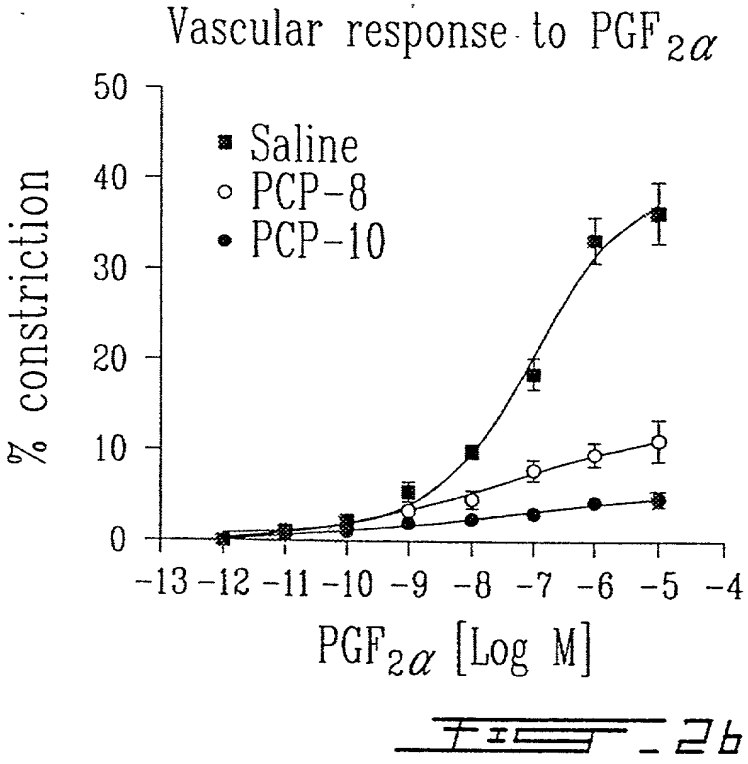


Fig - 2a


Vascular response to $PGF_{2\alpha}$
% constriction
50
40
30
20
10
0
Saline
PCP-8
PCP-10
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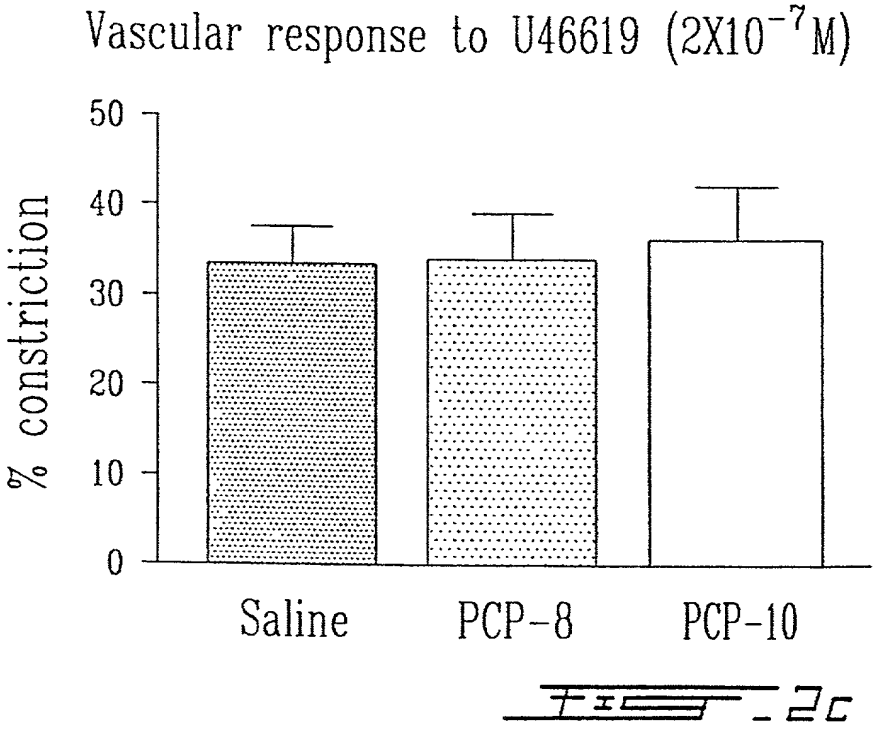
$PGF_{2\alpha}$ [Log M]

Fig 2b


Vascular response to U46619 ($2X10^{-7}M$)
% constriction
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10
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PCP-10

Fig 2c

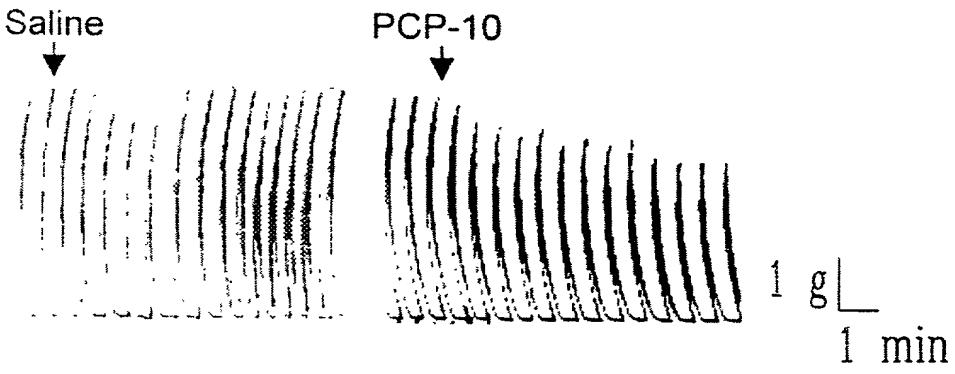
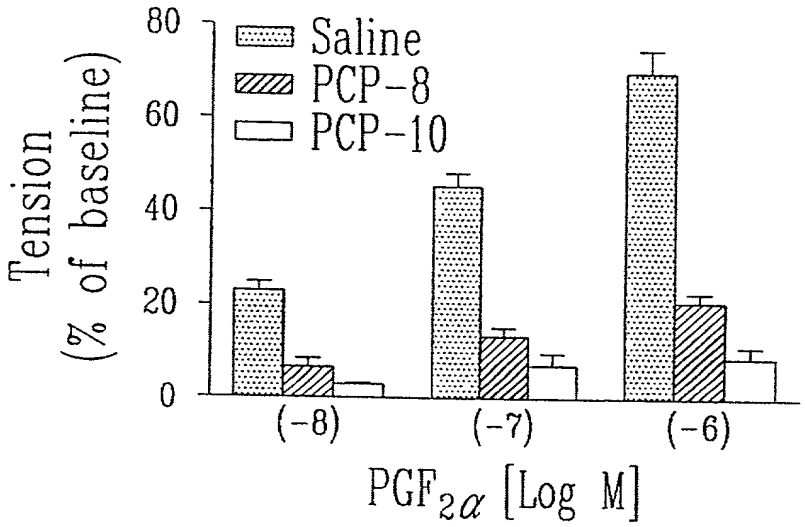


Fig 3a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Saline
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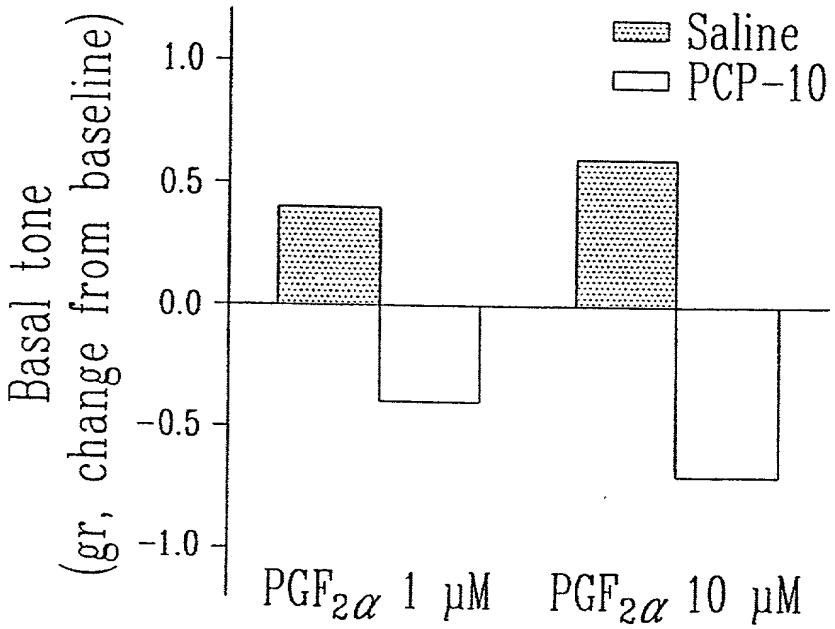
PCP-10
(-8)
(-7)
(-6)
PGF2α [Log M]

Fig 3b


Basal tone
(gr, change from baseline)
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-1.0
Saline
PCP-10
PGF2α 1 μM
PGF2α 10 μM

Fig 4

SEQUENCE LISTING

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PERI, Krishna G.

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Docket No. 2861-4003

COMBINED DECLARATION AND POWER OF ATTORNEY FOR
ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL
DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART APPLICATION

As below named inventors, we hereby declare that:

Our residence, post office address and citizenship are as stated below next to our names,

We believe we are the original, first and sole inventors (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

G PROTEIN-COUPLED RECEPTOR ANTAGONISTS
the specification of which

a. [ ] is attached hereto

b. [**X**] was filed on March 16, 2001 as application Serial No. 09/787,334 and was amended on _____. (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

c. [**X**] was described and claimed in International Application No. PCT/CA99/00844 filed on September 15, 1999 and as amended on _____. (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby specify the following as the correspondence address to which all communications about this application are to be directed:

SEND CORRESPONDENCE TO: MORGAN & FINNEGAN, L.L.P
345 Park Avenue
New York, N.Y. 10154

DIRECT TELEPHONE CALLS TO: Mary J. Morry, Esq.
(212) 758-4800

[ ] I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) or under § 365(b) of any foreign application(s) for patent or inventor's certificate or under § 365(a) of any PCT international application(s) designating at least one country other than the U.S. listed below and also have identified below such foreign application(s) for patent or inventor's certificate or such PCT international application(s) filed by me on the same subject matter having a filing date within twelve (12) months before that of the application on which priority is claimed:

[ ] The attached 35 U.S.C. § 119 claim for priority for the application(s) listed below forms a part of this declaration.

Country/PCT	Application Number	Date of filing (day, month, yr)	Date of Issue (day, month, yr)	Priority Claimed
				[ ] YES [ ] NO
				[ ] YES [ ] NO
				[ ] YES [ ] NO

[ ] I hereby claim the benefit under 35 U.S.C. § 119(e) of any U.S. provisional application(s) listed below.

Provisional Application No.	Date of Filing (day, month, yr)

ADDITIONAL STATEMENTS FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART OR PCT INTERNATIONAL APPLICATION(S) (DESIGNATING THE U.S.)

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or under § 365(c) of any PCT international application(s) designating the U.S. listed below.

US/PCT Application Serial No.	Filing Date	Status (patented, pending, abandoned)/ U.S. application no. assigned (For PCT)
09/154,627	17 September 1998	
PCT/CA99/00844	15 September 1999	

[ ] In this continuation-in-part application, insofar as the subject matter of any of the claims of this application is not disclosed in the above listed prior United States or PCT international application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or Imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Docket No. 2861-4003

I hereby appoint the following attorneys and/or agents with full power of substitution and revocation, to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith: John A. Diaz (Reg. No. 19,550), John C. Vassil (Reg. No. 19,098), Alfred P. Ewert (Reg. No. 19,887), David H. Pfeffer (Reg. No. 19,825), Harry C. Marcus (Reg. No. 22,390), Robert E. Paulson (Reg. No. 21,046), Stephen R. Smith (Reg. No. 22,615), Kurt E. Richter (Reg. No. 24,052), J. Robert Dailey (Reg. No. 27,434), Eugene Moroz (Reg. No. 25,237), John F. Sweeney (Reg. No. 27,471), Arnold I. Rady (Reg. No. 26,601), Christopher A. Hughes (Reg. No. 26,914), William S. Feiler (Reg. No. 26,728), Joseph A. Calvaruso (Reg. No. 28,287), James W. Gould (Reg. No. 28,859), Richard C. Komson (Reg. No. 27,913), Israel Blum (Reg. No. 26,710), Bartholomew Verdirame (Reg. No. 28,483), Maria C.H. Lin (reg. No. 29,323), Joseph A. DeGirolamo (Reg. No. 28,595), Michael P. Dougherty (Reg. No. 32,730), Seth J. Atlas (Reg. No. 32,454), Andrew M. Riddles (Reg. No. 31,657), Bruce D. DeRenzi (Reg. No. 33,676), Michael M. Murray (Reg. No. 32,537), Mark J. Abate (Reg. No. 32,527), John T. Gallagher (Reg. No. 35,516), Steven F. Meyer (Reg. No. 35,613), Kenneth H. Sonnenfeld (Reg. No. 33,285), Tony V. Pezzano (Reg. No. 38,271), Andrea L. Wayda (Reg. No. 43,979) and Walter G. Hanchuk Reg. No. (35,179) of Morgan & Finnegan, L.L.P. whose address is: 345 Park Avenue, New York, New York, 10154; and Michael S. Marcus (Reg. No. 31,727) and John E. Hoel (Reg. No. 26,279) of Morgan & Finnegan, L.L.P., whose address is 1775 Eye Street, Suite 400, Washington, D.C. 20006.

33

[ ] I hereby authorize the U.S. attorneys and/or agents named hereinabove to accept and follow instructions from _____ as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and/or agents and me. In the event of a change in the person(s) from whom instructions may be taken I will so notify the U.S. attorneys and/or agents hereinabove.

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Inventor's signature* Sylvain Chemtob 01-06-01
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Full name of third joint inventor, if any _____

Inventor's signature* _____
date

Residence _____

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[ ] ATTACHED IS/ARE ADDED PAGE(S) TO COMBINED DECLARATION AND POWER OF ATTORNEY FORM FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

* Before signing this declaration, each person signing must:

1. Review the declaration and verify the correctness of all information therein; and

2. Review the specification and the claims, including any amendments made to the claims.

After the declaration is signed, the specification and claims are not to be altered.

To the inventor(s):

The following are cited in or pertinent to the declaration attached to the accompanying application:

Title 37, Code of Federal Regulation, § 1.56

Duty to disclose information material to patentability.

(a) A patent by its very nature is affect with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in patent was cited by the Office or submitted to the Office in the manner prescribed by §§1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

Title 35, U.S. Code § 101

Inventions patentable

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Title 35 U.S. Code § 102

Conditions for patentability; novelty and loss of right to patent

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent,

(b) the invention was patented or described in a printed publication in this or foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States, or

(c) he has abandoned the invention, or

(d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate field more than twelve months before the filing of the application in the United States, or

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or

(f) he did not himself invent the subject matter sought to be patented, or

(g) before the applicant's invention thereof the invention was made in this country by another had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other …

Title 35, U.S. Code § 103

Conditions for patentability; non-obvious subject matter

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having

ordinary skill in the art to which said matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Title 35, U.S. Code § 112 (in part)

Specification

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms also enable any person skilled in the art to which it pertains, or with which it is mostly nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Title 35, U.S. Code § 119

Benefit of earlier filing date in foreign country; right of priority

An application for patent for an invention filed in this country by any person who has, or whose legal representatives or assigns have, previously regularly filed an application for a patent for the same invention in a foreign country which affords similar privileges in the case of applications filed in the United States or to citizens of the United States, shall have the same effect as the same application would have if filed in this country on the date on which the application for patent for the same invention was first filed in such foreign country, if the application in this country is filed within twelve months from the earliest date on which such foreign application was filed; but no patent shall be granted on any application for patent for an invention which had been patented or described in a printed publication in any country more than one year before the date of he actual filing of the application in this country, or which had been in public use or on sale in this country more than one year prior to such filing.

Title 35, U.S. Code § 120

Benefit or earlier filing date in the United States

An application for patent for an invention disclosed in the manner provided by the first paragraph of section 112 of this title in an application previously filed in the United States, or as provided by section 363 of this title, which is filed by an inventor or inventors named in the previously filed application shall have the same effect, as to such invention, as though filed on the date of the prior application, if filed before the patenting or abandonment of or termination of proceedings on the first application or an application similarly entitled to the benefit of the filing date of the first application and if it contains or is amended to contain a specific reference to the earlier filed application.

Please read carefully before signing the Declaration attached to the accompanying Application.

If you have any questions, please contact Morgan & Finnegan, L.L.P.

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